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Dated

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1. Your reference

ACC/JLD/BM45323

2. Patent application number

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3. Full name, address and postcode of the or of each applicant (underline all surnames)

SmithKline Beecham Biologicals s.a.
rue de l'Institut 89, B-1330 Rixensart, Belgium

Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

Belgium

0573 117001

4. Title of the invention

NOVEL COMPOUNDS

5. Name of your agent (*if you have one*)

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6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and (*if you know it*) the or each application number

Country	Priority application number (<i>if you know it</i>)	Date of filing (<i>day / month / year</i>)
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (<i>day / month / year</i>)
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (*Answer yes if:*

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is named as an applicant, or
 - c) any named applicant is a corporate body
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BASB030

FIELD OF THE INVENTION

This invention relates to newly identified polynucleotides and polypeptides, and their production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, the invention relates to polynucleotides and polypeptides of the PilQ (fimbrial assembly) family, as well as their variants, hereinafter referred to as "BASB030," "BASB030 polynucleotide(s)," and "BASB030 polypeptide(s)" as the case may be

BACKGROUND OF THE INVENTION

Neisseria meningitidis (meningococcus) is a Gram negative bacterium frequently isolated from the human upper respiratory tract. It occasionally causes invasive bacterial diseases such as bacteremia and meningitis. The incidence of meningococcal disease shows geographical seasonal and annual differences (Schwartz, B., Moore, P.S., Broome, C.V. (1989) Global epidemiology of meningococcal disease. Clin. Microbiol. Rev. 2 (Supplement), S18-S24). Most disease in temperate countries is due to strains of serogroup B and varies in incidence from 1-10/100,000/year total population sometimes reaching higher values (Kaczmarek, E.B. (1997). Meningococcal disease in England and Wales : 1995. Commun. Dis. Rep. Rev. 7 : R55-9; Scholten, R.J.P.M., Bijlmer, H.A., Poolman, J.T. et al. (1993) Meningococcal disease in the Netherlands, 1958-1990. Steady increase in the incidence rate since 1982 partially caused by new serotypes and subtypes of *Neisseria meningitidis*. Clin. Infect. Dis. 16 : 237-246; Cruz, C., Pavez, G., Aguilar, E., et al. (1990) Serotype-specific outbreak of group B meningococcal disease in Iquique, Chile Epidemiol. Infect. 105 : 119-126).

Epidemics dominated by serogroup A meningococci, mostly in central Africa, are encountered, sometimes reaching levels up to 1000/100.000/year (Schwartz, B., Moore, P.S., Broome, C.V. (1989) Global epidemiology of meningococcal disease. Clin. Microbiol. Rev. 2 (Supplement), S18-S24). Nearly all cases as a whole of meningococcal disease are caused by serogroup A, B, C, W-135 and Y meningococci and a tetravalent A, C, W-135, Y polysaccharide vaccine is available (Armand, J., Arminjon, F., Mynard, M.C., Lafaix, C., (1982). Tetravalent meningococcal polysaccharide vaccine group A, C, Y, W-135 : Clinical and Serological evaluation. J. Biol. Stand. 10 : 335-339).

The polysaccharide vaccines are currently being improved by way of chemical conjugating them to carrier proteins (Lieberman, J.M., Chiu, S.S., Wong, V.K., et al. (1996). Safety and immunogenicity

disease (Ross, S.C., Rosenthal P.J., Berberic, H.M., Densen, P. (1987). Killing of *Neisseria meningitidis* by human neutrophils : implications for normal and complement deficient individuals. J. Infect. Dis. 155 : 1266-1275).

The frequency of *Neisseria meningitidis* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Neisseria meningitidis* strains which are resistant to some or all of the standard antibiotics. This phenomenon has created an unmet medical need and demand for new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

Moreover, the drug discovery process is currently undergoing a fundamental revolution as it embraces "functional genomics," that is, high throughput genome- or gene-based biology. This approach is rapidly superseding earlier approaches based on "positional cloning" and other methods. Functional genomics relies heavily on the various tools of bioinformatics to identify gene sequences of potential interest from the many molecular biology databases now available as well as from other sources. There is a continuing and significant need to identify and characterize further genes and other polynucleotides sequences and their related polypeptides, as targets for drug discovery.

Clearly, there exists a need for polynucleotides and polypeptides, such as the BASB030 embodiments of the invention, that have a present benefit of, among other things, being useful to screen compounds for antibiotic activity. Such factors are also useful to determine their role in pathogenesis of infection, dysfunction and disease. There is also a need for identification and characterization of such factors and their antagonists and agonists to find ways to prevent, ameliorate or correct such infection, dysfunction and disease.

Certain of the polypeptides of the invention possess significant amino acid sequence homology to a known *N.gonorrhoeae* PilQ protein.

SUMMARY OF THE INVENTION

The present invention relates to BASB030, in particular BASB030 polypeptides and BASB030 polynucleotides, recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including the treatment of microbial diseases, amongst others. In a further aspect, the invention relates to methods for identifying agonists and antagonists using the materials provided by the invention, and for treating microbial infections and conditions associated with such infections with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for

ATTATCACAAACAGCCGGCAACTGGGAACTCGTCAACAAATCCGCCGCGCCCGGATACTTT
 ACCTTCCAAGTCCTGCCGAAAAACAAACCTCGAGTCAGGCGGCGTGAACAATGCGCCC
 AAAACCTTCACAGGCCGGAAAAATCTCCCTTGACTTCCAAGATGTCGAAATCCGCACCATC
 CTGCAGATTTTGGCAAAGAATCCGGGATGAACATTGTTGCCAGCGACTCCGTCAACGGC
 5 AAAATGACCCTCTCCCTCAAAGACGTACCTTGGGATCAGGCTTTGGATTGTTATGCAG
 GCACGCAACCTCGATATGCGCCAACAAGGGAACATCGTCAACATCGCGCCCCGCGACGAG
 CTGCTTGCCAAAGACAAAGCCTTCTTACAGGCGGAAAAAGACATTGCCGATCTAGGCGCG
 CTGTATTCACAAAACCTTCCAATTGAAATACAAAATGTGGAAGAATTCCGCAGCATCCTG
 CGTTTGGACAATGCCGACACAACCGGAAACCGCAATACGCTTGTCAGCGGCAGGGGCAGC
 10 GTGCTGATCGATCCCGCCACCAATACCCTGATTGTTACCGATACCCGCAGCGTCATCGAA
 AAATTCCGCAAACCTGATTGACGAATTGGACGTACCCGCGCAACAAGTGATGATTGAGGCG
 CGTATCGTCGAAGCGGCAGACGGCTTCTCGCGCGATTGTTGGGCGTTAAATTGCGCGCGACA
 GGCAAGAAAAAGCTGAAAAATGATACAAGCGCATTCGGCTGGGGGGTAAACTCCGGCTTC
 GGCGGCGACGATAAATGGGGGGCCGAAACCAAATCAACCTGCCGATTACCGCTGCCGCA
 15 AACAGCATTTTCGCTGGTGCGCGGATTTCCCTCCGGTGCCTTGAATTTGGAATTGTCCGCA
 TCCGAATCGCTTTCAAAAACCAAACGCTTGCCAATCCGCGCGTGCTGACCCAAAACCGC
 AAAGAGGCCAAAATCGAATCCGGTTACGAAATTCCTTTCACCGTAACCTCAATCGCGAAC
 GGCGGCAGCAGCACGAACACGGAACCTCAAAAAAGCCGTCTTGGGGCTGACCGTTACGCCG
 AACATCACGCCCCGACGGCCAAATCATTATGACCGTCAAAATCAACAAGGACTCGCCTGCG
 20 CAATGTGCCTCCGGTAATCAGACGATCCTGTGTATTTGACCAAAAACCTGAATACGCAG
 GCTATGGTTGAAAACGGCGGCACATTGATTGTGCGCGGTATTTATGAAGAAGACAACGGC
 AATACGCTGACCAAAGTCCCCCTGTTGGGCGACATCCCCGTTATCGGCAACCTCTTTAAA
 ACACGCGGGAAAAAAACCGACCGCCGCGAACTGCTGATTTTCATTACCCCGAGGATTATG
 GGTACGGCCGGCAACAGCCTGCGCTATTGA-3'

25

(B) *Neisseria meningitidis* BASB030 polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO:2].

NH₂->BASB030

MNTKLTKIISGLFVATAAFQTASAGNITDIKVSSLPNKQKIVKVSFDKEIVNPTGFVTSS
 30 PARIALDFEQTGISMDQQVLEYADPLLSKISAAQNSSRARLVLNLNKPQYNTTEVRGNKV
 WIFINESDDTVSAPARPAVKAAPAAPAKQQGCRTVYQVRSIRIQTLYPGKTTAAAPFTES

In one aspect of the invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the *Neisseria meningitidis* Serogroup B strain, which polypeptide is contained in the deposited strain. Further provided by the invention are BASB030 polynucleotide sequences in the deposited strain, such as DNA and RNA, and amino acid sequences encoded thereby. Also provided by the invention are BASB030 polypeptide and polynucleotide sequences isolated from the deposited strain.

Polypeptides

BASB030 polypeptide of the invention is substantially phylogenetically related to other proteins of the PilQ (fimbrial assembly) family.

In one aspect of the invention there are provided polypeptides of *Neisseria meningitidis* referred to herein as "BASB030" and "BASB030 polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

Among the particularly preferred embodiments of the invention are variants of BASB030 polypeptide encoded by naturally occurring alleles of the BASB030 gene.

The present invention further provides for an isolated polypeptide which:

- (a) comprises or consists of an amino acid sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2;
- (b) a polypeptide encoded by an isolated polynucleotide comprising or consisting of a polynucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1;
- (c) a polypeptide encoded by an isolated polynucleotide comprising or consisting of a polynucleotide sequence encoding a polypeptide which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2.

The polypeptides of the invention include a polypeptide of Table 1 [SEQ ID NO:2] (in particular the mature polypeptide) as well as polypeptides and fragments, particularly those which have the biological activity of BASB030, and also those which have at least 70% identity to a polypeptide of Table 1 [SEQ ID NO:1] or the relevant portion, preferably at least 80% identity to a

functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO: 2, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO: 2.

Also preferred are biologically active fragments which are those fragments that mediate activities of BASB030, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those fragments that are antigenic or immunogenic in an animal, especially in a human. Particularly preferred are fragments comprising receptors or domains of enzymes that confer a function essential for viability of *Neisseria meningitidis* or the ability to initiate, or maintain cause Disease in an individual, particularly a human.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.

In addition to the standard single and triple letter representations for amino acids, the term "X" or "Xaa" may also be used in describing certain polypeptides of the invention. "X" and "Xaa" mean that any of the twenty naturally occurring amino acids may appear at such a designated position in the polypeptide sequence.

Polynucleotides

It is an object of the invention to provide polynucleotides that encode BASB030 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB030.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB030 polypeptides comprising a sequence set out in Table 1 [SEQ ID NO:1] which includes a full length gene, or a variant thereof. The Applicants believe that this full length gene is essential to the growth and/or survival of an organism which possesses it, such as *Neisseria meningitidis*.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB030 polypeptides and polynucleotides, particularly *Neisseria*

Moreover, each DNA sequence set out in Table 1 [SEQ ID NO:1] contains an open reading frame encoding a protein having about the number of amino acid residues set forth in Table 1 [SEQ ID NO:2] with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art. The polynucleotide of SEQ ID NO: 1, between nucleotide number 1 and the stop codon which begins at nucleotide number 2308 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- (a) a polynucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1;
- (b) a polynucleotide sequence encoding a polypeptide which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO: 1 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in Table 1 [SEQ ID NO:1]. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that

genus. A polynucleotide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB030 having an amino acid sequence set out in Table 1 [SEQ ID NO:2]. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of Table 1 [SEQ ID NO:2]. Fragments of a polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB030 variants, that have the amino acid sequence of BASB030 polypeptide of Table 1 [SEQ ID NO:2] in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of BASB030 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical over their entire length to a polynucleotide encoding BASB030 polypeptide having an amino acid sequence set out in Table 1 [SEQ ID NO:2], and polynucleotides that are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding BASB030 polypeptide and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.



sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

5 For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such
10 inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA
15 sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein
20 having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

Vectors, Host Cells, Expression Systems

25 The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

30 Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems which comprise a polynucleotide or polynucleotides of the present invention, to host cells which are

environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic, Prognostic, Serotyping and Mutation Assays

This invention is also related to the use of BASB030 polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of BASB030 polynucleotides and/or polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the BASB030 gene or protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from any of these sources, particularly DNA or RNA, may be used directly for detection or may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled BASB030 polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched duplexes by DNase or RNase digestion, for DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in

particular location on an organism's chromosome, particularly to a *Neisseria meningitidis* chromosome. The mapping of relevant sequences to chromosomes according to the present invention may be an important step in correlating those sequences with pathogenic potential and/or an ecological niche of an organism and/or drug resistance of an organism, as well as the essentiality of the gene to the organism. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data may be found on-line in a sequence database. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through known genetic methods, for example, through linkage analysis (coinheritance of physically adjacent genes) or mating studies, such as by conjugation.

The differences in a polynucleotide and/or polypeptide sequence between organisms possessing a first phenotype and organisms possessing a different, second different phenotype can also be determined. If a mutation is observed in some or all organisms possessing the first phenotype but not in any organisms possessing the second phenotype, then the mutation is likely to be the causative agent of the first phenotype.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose, PCR. As an example, PCR primers complementary to a polynucleotide encoding BASB030 polypeptide can be used to identify and analyze mutations.

These primers may be used for, among other things, amplifying BASB030 DNA and/or RNA isolated from a sample derived from an individual, such as a bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual, such that the polynucleotide may then be subject to various techniques for elucidation of the polynucleotide sequence. In this way, mutations in the polynucleotide sequence may be detected and used to diagnose and/or prognose the infection or its stage or course, or to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing, disease, preferably bacterial infections, more preferably infections caused by *Neisseria meningitidis*, comprising determining from a sample derived from an individual, such as a bodily material, an increased level of

carry a promoter or portion of a gene normally upregulated during infection. Sequencing upstream of the recombinase gene allows identification of the up regulated gene.

RT-PCR may also be used to analyze gene expression patterns. For RT PCR using the polynucleotides of the invention, messenger RNA is isolated from bacterial infected tissue, e.g., 48 hour murine lung infections, and the amount of each mRNA species assessed by reverse transcription of the RNA sample primed with random hexanucleotides followed by PCR with gene specific primer pairs. The determination of the presence and amount of a particular mRNA species by quantification of the resultant PCR product provides information on the bacterial genes which are transcribed in the infected tissue. Analysis of gene transcription can be carried out at different times of infection to gain a detailed knowledge of gene regulation in bacterial pathogenesis allowing for a clearer understanding of which gene products represent targets for screens for antibacterials. Because of the gene specific nature of the PCR primers employed it should be understood that the bacterial mRNA preparation need not be free of mammalian RNA. This allows the investigator to carry out a simple and quick RNA preparation from infected tissue to obtain bacterial mRNA species which are very short lived in the bacterium (in the order of 2 minute halflives). Optimally the bacterial mRNA is prepared from infected murine lung tissue by mechanical disruption in the presence of TRIzol (GIBCO-BRL) for very short periods of time, subsequent processing according to the manufacturers of TRIzol reagent and DNAase treatment to remove contaminating DNA. Preferably the process is optimized by finding those conditions which give a maximum amount of *Neisseria meningitidis* 16S ribosomal RNA as detected by probing Northernblots with a suitably labeled sequence specific oligonucleotide probe. Typically a 5' dye labeled primer is used in each PCR primer pair in a PCR reaction which is terminated optimally between 8 and 25 cycles. The PCR products are separated on 6% polyacrylamide gels with detection and quantification using GeneScanner (manufactured by ABI).

Gridding and Polynucleotide Subtraction

Methods have been described for obtaining information about gene expression and identity using so called "high density DNA arrays" or grids. See, e.g., M. Chee *et al.*, *Science*, 274:610-614 (1996) and other references cited therein. Such gridding assays have been employed to identify certain novel gene sequences, referred to as Expressed Sequence Tags (EST) (Adams *et al.*, *Science*, 252:1651-1656 (1991)). A variety of techniques have also been described for identifying particular gene sequences on the basis of their gene products. For example, see International Patent Application No. WO91/07087, published May 30, 1991. In

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-BASB030 or from naive libraries (McCafferty, *et al.*, (1990), *Nature* 348, 552-554; Marks, *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*, (1991) *Nature* 352: 628).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or polynucleotides by, for example, affinity chromatography.

Thus, among others, antibodies against BASB030-polypeptide or BASB030-polynucleotide may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

A polypeptide or polynucleotide of the invention, such as an antigenically or immunologically equivalent derivative or a fusion protein of the polypeptide is used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein for example bovine serum albumin, keyhole limpet haemocyanin or tetanus toxoid. Alternatively, a multiple antigenic polypeptide comprising multiple copies of the polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complementarity determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones *et al.* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) *Biotechnology* 9, 266-273.

In accordance with an aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization. Among the particularly preferred embodiments of the invention are naturally occurring allelic variants of BASB030 polynucleotides and polypeptides encoded thereby.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff *et al.*, *Hum Mol Genet* (1992) 1: 363, Manthorpe *et al.*, *Hum. Gene Ther.* (1983) 4: 419), delivery

and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring BASB030 polypeptide and/or polynucleotide activity in the mixture, and comparing the BASB030 polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and BASB030 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB030 polypeptides or polynucleotides, particularly those compounds that are bacteristatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB030 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB030 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB030 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of BASB030 polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal

at low site density such that covalently bound molecules will be monomeric. Solution protein can then be passed over the BASB030 polypeptide-coated surface and specific binding can be detected in real-time by monitoring the change in resonance angle caused by a change in local refractive index. This technique can be used to characterize the effect of small molecules on kinetic rates and equilibrium binding constants for BASB030 polypeptide self-association as well as an association of BASB030 polypeptide and another polypeptide or small molecule.

A scintillation proximity assay may be used to characterize the interaction between an association of BASB030 polypeptide with another BASB030 polypeptide or a different polypeptide. BASB030 polypeptide can be coupled to a scintillation-filled bead. Addition of radio-labeled BASB030 polypeptide results in binding where the radioactive source molecule is in close proximity to the scintillation fluid. Thus, signal is emitted upon BASB030 polypeptide binding and compounds that prevent BASB030 polypeptide self-association or an association of BASB030 polypeptide and another polypeptide or small molecule will diminish signal.

ICS biosensors have been described by AMBRI (Australian Membrane Biotechnology Research Institute). They couple the self-association of macromolecules to the closing of gramicidin-facilitated ion channels in suspended membrane bilayers and hence to a measurable change in the admittance (similar to impedance) of the biosensor. This approach is linear over six decades of admittance change and is ideally suited for large scale, high through-put screening of small molecule combinatorial libraries.

In other embodiments of the invention there are provided methods for identifying compounds which bind to or otherwise interact with and inhibit or activate an activity or expression of a polypeptide and/or polynucleotide of the invention comprising: contacting a polypeptide and/or polynucleotide of the invention with a compound to be screened under conditions to permit binding to or other interaction between the compound and the polypeptide and/or polynucleotide to assess the binding to or other interaction with the compound, such binding or interaction preferably being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the polypeptide and/or polynucleotide with the compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an activity or expression of the polypeptide and/or polynucleotide by detecting the presence or absence of a signal generated from the binding or interaction of the compound with the polypeptide and/or polynucleotide.

Another example of an assay for BASB030 agonists is a competitive assay that combines BASB030 and a potential agonist with BASB030-binding molecules, recombinant BASB030

binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. BASB030 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB030 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing BASB030-induced activities, thereby preventing the action or expression of BASB030 polypeptides and/or polynucleotides by excluding BASB030 polypeptides and/or polynucleotides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of BASB030.

Other examples of potential polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Certain of the polypeptides of the invention are biomimetics, functional mimetics of the natural BASB030 polypeptide. These functional mimetics may be used for, among other things, antagonizing the activity of BASB030 polypeptide or as an antigen or immunogen in a manner described elsewhere herein. Functional mimetics of the polypeptides of the invention include but are not limited to truncated polypeptides. For example, preferred functional mimetics include, a polypeptide comprising the polypeptide sequence set forth in SEQ ID NO:2 lacking 20, 30, 40, 50, 60, 70 or 80 amino- or carboxy-terminal amino acid residues, including fusion proteins comprising one or more of these truncated sequences. Polynucleotides encoding each of these functional

thereof an inhibitor compound (antagonist) as herein described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function and/or expression of the polypeptide and/or polynucleotide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of the polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous polypeptide and/or polynucleotide may be administered. Typical examples of such competitors include fragments of the BASB030 polypeptide and/or polypeptide.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

In still another approach, expression of the gene encoding endogenous BASB030 polypeptide can be inhibited using expression blocking techniques. This blocking may be targeted against any step in gene expression, but is preferably targeted against transcription and/or translation. An examples of a known technique of this sort involve the use of antisense sequences, either internally generated or separately administered (see, for example, O'Connor, *J Neurochem* (1991) 56:560 in *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988)). Alternatively, oligonucleotides which form triple helices with the gene can be supplied (see, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360). These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other

polypeptide and/or polynucleotide to an organism to raise an immunological response against a bacteria, especially a *Neisseria meningitidis* bacteria.

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the individual with
5 BASB030 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Neisseria meningitidis* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an
10 individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB030 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB030 polynucleotide and/or polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic
15 T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

20 A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a BASB030 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB030 polynucleotide and/or polypeptide encoded therefrom and/or comprises
25 DNA and/or RNA which encodes and expresses an antigen of said BASB030 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A BASB030 polypeptide or a fragment thereof may be fused with co-protein or chemical
30 moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Hemophilus*

and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include
5 adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

While the invention has been described with reference to certain BASB030 polypeptides and polynucleotides, it is to be understood that this covers fragments of the naturally occurring
10 polypeptides and polynucleotides, and similar polypeptides and polynucleotides with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

Compositions, kits and administration

In a further aspect of the invention there are provided compositions comprising a BASB030
15 polynucleotide and/or a BASB030 polypeptide for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising a polynucleotide and/or a polypeptides discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for
20 use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode
25 of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

30 The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

5 In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

10 The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially *Neisseria meningitidis* wound infections.

15 Many orthopedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

20 In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1µg/ml to 10mg/ml for bathing of wounds or indwelling devices.

25 A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

30 Sequence Databases, Sequences in a Tangible Medium, and Algorithms

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar

comprising the sequence of a polynucleotide sequence of the invention; a data set representing a polynucleotide sequence encoding a polypeptide sequence comprising the sequence of a polypeptide sequence of the invention. The computer readable medium can be any composition of matter used to store information or data, including, for example, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks.

Also provided by the invention are methods for the analysis of character sequences or strings, particularly genetic sequences or encoded genetic sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and similarity analysis, RNA structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, nucleic acid base trimming, and sequencing chromatogram peak analysis.

A computer based method is provided for performing homology identification. This method comprises the steps of providing a polynucleotide sequence comprising the sequence a polynucleotide of the invention in a computer readable medium; and comparing said polynucleotide sequence to at least one polynucleotide or polypeptide sequence to identify homology.

A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and comparing said polypeptide sequence to at least one polynucleotide or polypeptide sequence to identify homology.

A computer based method is still further provided for polynucleotide assembly, said method comprising the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and screening for at least one overlapping region between said first polynucleotide sequence and a second polynucleotide sequence.

A further embodiment of the invention provides a computer based method for performing homology identification, said method comprising the steps of: providing a polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and comparing said polynucleotide sequence to at least one polynucleotide or polypeptide sequence to identify homology.

A further embodiment of the invention provides a computer based method for performing homology identification, said method comprising the steps of: providing a polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and

sequence comprising the sequence of SEQ ID NO. 1 in a computer readable medium; and screening for at least one overlapping region between said first polynucleotide sequence and a second polynucleotide sequence.

5 A further embodiment of the invention provides a computer based method for performing homology identification, said method comprising the steps of: providing a polynucleotide sequence comprising the sequence of SEQ ID NO. 1 in a computer readable medium; and comparing said polynucleotide sequence to at least one polynucleotide or polypeptide sequence to identify homology.

10 A further embodiment of the invention provides a computer based method for performing homology identification, said method comprising the steps of: providing a polypeptide sequence comprising the sequence of SEQ ID NO. 2 in a computer readable medium; and comparing said polypeptide sequence to at least one polynucleotide or polypeptide sequence to identify homology.

15 A further embodiment of the invention provides a computer based method for polynucleotide assembly, said method comprising the steps of: providing a first polynucleotide sequence comprising the sequence of SEQ ID NO. 1 in a computer readable medium; and screening for at least one overlapping region between said first polynucleotide sequence and a second polynucleotide sequence.

20 All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner
25 described above for publications and references.

GLOSSARY

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

30 "Antibody(ies)" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are
 5 designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X
 10 program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

- 1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)
- 15 Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

- A program useful with these parameters is publicly available as the "gap" program from Genetics
 20 Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

- 1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

- 25 Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

- 30 A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be

$$n_n \leq x_n - (x_n \circ y),$$

wherein n_n is the number of nucleic acid alterations, x_n is the total number of nucleic acids in
 5 SEQ ID NO:1, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., \circ is the symbol
 for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down
 to the nearest integer prior to subtracting it from x_n .

(2) Polypeptide embodiments further include an isolated polypeptide comprising a
 polypeptide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide
 10 reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the
 reference sequence of SEQ ID NO: 2 or may include up to a certain integer number of amino acid
 alterations as compared to the reference sequence, wherein said alterations are selected from the
 group consisting of at least one amino acid deletion, substitution, including conservative and non-
 conservative substitution, or insertion, and wherein said alterations may occur at the amino- or
 15 carboxy-terminal positions of the reference polypeptide sequence or anywhere between those
 terminal positions, interspersed either individually among the amino acids in the reference
 sequence or in one or more contiguous groups within the reference sequence, and wherein said
 number of amino acid alterations is determined by multiplying the total number of amino acids in
 SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that
 20 product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \circ y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ
 25 ID NO:2, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for
 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and \circ is the symbol for the multiplication
 operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer
 prior to subtracting it from x_a .

By way of example, a polypeptide sequence of the present invention may be identical to
 30 the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to
 a certain integer number of amino acid alterations as compared to the reference sequence such
 that the percent identity is less than 100% identity. Such alterations are selected from the group

- "Organism(s)" means a (i) prokaryote, including but not limited to, a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*, *Enterobacter*, *Yersinia*, *Fancisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*, *Branhamella*, *Actinobacillus*, *Streptobacillus*,
 5 *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Kleibsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not limited to, a member of the species or group, Group A *Streptococcus*, Group B *Streptococcus*, Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*,
 10 *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*, *Mycobacterium leprae*, *Actinomycetes israelii*, *Listeria monocytogenes*, *Bordetella pertussis*,
 15 *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia pestis*, *Kleibsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenterii*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Francisella tularensis*, *Brucella abortis*,
 20 *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii* and *Chlamydia trachomatis*, (ii) an archaeon, including but not limited to *Archaeobacter*, and (iii) a unicellular or filamentous eukaryote, including but not limited to, a protozoan, a fungus, a member of the genus *Saccharomyces*, *Kluveromyces*, or *Candida*, and a member of the species *Saccharomyces cerevisiae*, *Kluveromyces lactis*, or *Candida albicans*.
 25

- "Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA,
 30 and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in

carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F.,

- 5 Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663: 48-62 (1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and
- 10 branched circular polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.


"Recombinant expression system(s)" refers to expression systems or portions thereof or polynucleotides of the invention introduced or transformed into a host cell or host cell lysate for the production of the polynucleotides and polypeptides of the invention.

- 15 "Subtraction set" is one or more, but preferably less than 100, polynucleotides comprising at least one polynucleotide of the invention

- "Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference
- 20 polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusion proteins and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally,
- 25 differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. The present invention also includes include variants of each of the polypeptides of the
- 30 invention, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.


What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence which has at least 70% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.
- 5 2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity.
3. The polypeptide as claimed in claim 1 comprising the amino acid sequence of SEQ ID NO:2.
- 10 4. The isolated polypeptide of SEQ ID NO:2.
5. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 70% identity to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.
- 15 6. An isolated polynucleotide comprising a nucleotide sequence that has at least 70% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.
- 20 7. An isolated polynucleotide which comprises a nucleotide sequence which has at least 70% identity to that of SEQ ID NO:1 over the entire length of SEQ ID NO:1; or a nucleotide sequence complementary to said isolated polynucleotide.
- 25 8. The isolated polynucleotide as claimed in any one of claims 5 to 7 in which the identity is at least 95%.
9. An isolated polynucleotide selected from:
 - (a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2;
 - 30 (b) the polynucleotide of SEQ ID NO:1; and
 - (c) a polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof; or a nucleotide sequence complementary to said isolated polynucleotide

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- (a) an agonist or antagonist to the polypeptide of claims 1 to 4;
 - (b) isolated polynucleotide of claims 5 to 9; or
 - (c) a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide of claim 1;

5 for use in therapy.

17. A process for diagnosing a disease or a susceptibility to a disease in an individual related to expression or activity of the polypeptide of claim 1 in an individual comprising:

- (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said
 - 10 polypeptide in the genome of said individual; and/or
 - (b) analyzing for the presence or amount of said polypeptide expression in a sample derived from said individual.
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